RESEARCH PAPER

The function of α- and β-adrenoceptors of the saphenous artery in caveolin-1 knockout and wild-type mice

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Background and purpose: Adrenoceptors can associate with cardiac caveolae. To investigate the function of vascular caveolae, adrenoceptor-mediated effects were compared in the saphenous artery of caveolin-1 knockout (cav-1KO) and wild-type (WT) mice.

Experimental approach: Electronmicroscopy was used to detect caveolae. Real-Time quantitative PCR was used for adrenoceptor subtypes. Catecholamine-evoked contractions and relaxations were studied in arterial segments.

Key results: Caveolae were found in arterial smooth muscle from WT but not from cav-1KO mice. Arterial mRNA levels for the adrenoceptors α_{1A}, α_{1B}, α_{1D}, β_1, β_2 and β_3 were similar in cav-1KO and WT. (-)-Noradrenaline contracted cav-1KO (-log EC_{50}M = 7.1) and WT (-log EC_{50}M = 7.3) arteries through prazosin-sensitive receptors. Maximum (-)-noradrenaline-evoked contractions were greater in cav-1KO than WT arteries. (-)-Isoprenaline relaxed WT arteries (-log EC_{50}M = 7.3) more potently than cav-1KO arteries (-log EC_{50}M = 6.8); the effects were antagonized partially and similarly by the β_2-selective antagonist ICI118551 (50 nM). The (-)-isoprenaline-evoked relaxation was partially antagonized by the β_1-adrenoceptor-selective antagonist CGP20712 (300 nM) in WT but not cav-1KO arteries. The β_3-adrenoceptor-selective antagonist L748337 (100nM) partially antagonized the relaxant effects of (-)-isoprenaline in cav-1KO but not in WT arteries. BRL37344 partially relaxed arteries through β_3-adrenoceptors in cav-1KO but not WT. The relaxant effects of BRL37344 were decreased by the NO synthase inhibitor ΩL-nitroarginine.

Conclusions and implications: The function of arterial α_1- and β_2-adrenoceptors is similar in cav-1KO and WT mice. β_1-adrenoceptor-mediated relaxation in WT is lost in cav-1KO and replaced by the appearance of β_3-adrenoceptors.

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Introduction

Cell membrane invaginations (Palade, 1953), coined caveolae (Yamada, 1955), contain the scaffolding protein caveolin that modulates the function of colocalized proteins. Caveolin-regulated proteins include α_1-adrenoceptors (Fujita *et al.*, 2001; Bucci *et al.*, 2004), β_1- and β_2-adrenoceptors (Schwencke *et al.*, 1999; Ostrom *et al.*, 2000; Rybin *et al.*, 2000) and adenylyl cyclase (Ostrom *et al.*, 2000, 2001, 2002). Mice with the disruption of the caveolin-1 gene (Razani *et al.*, 2002), the most ubiquitous of three caveolins, are available (Drab *et al.*, 2001; Razani *et al.*, 2001; Park *et al.*, 2003).

The scaffolding domain of caveolin-1 potentiates the constriction of murine aortic rings evoked by phenylephrine, presumably through inhibition of nitric oxide (NO) synthesis (Bucci *et al.*, 2000). Conversely, the vasoconstrictor effect of phenylephrine is blunted in the aorta from cav-1 knock-

out (cav-1KO) mice but acetylcholine-induced relaxation is enhanced through increased NO synthesis and release (Drab et al., 2001; Razani et al., 2001). Although the effects of phenylephrine are presumably mediated through α-adrenoceptors, the nature of these receptors and their interaction with noradrenaline in a small artery are unknown. We have therefore compared the function of α-adrenoceptors, activated by (−)-noradrenaline, in the saphenous artery of cav-1KO and wild-type (WT) mice, in the absence and presence of the α_1-adrenoceptor-selective antagonist prazosin.

β-Adrenoceptors appear associated with caveolae before but not after agonist treatment (Insel et al., 2005). Agonist stimulation has been found to cause a decline in the abundance of caveolar β_2-adrenoceptors but not of β_1-adrenoceptors in cardiomyocytes (Rybin et al., 2000). However, the role of caveolae in the vascular β-adrenoceptor function is unknown. We therefore investigated the function of β-adrenoceptor subtypes in the saphenous artery of cav-1KO and WT mice.

Methods

Mice
Black six mice (C57Black6, WT) and caveolin-1 knockout mice (cav-1KO), aged 9–18 months, were used. Cav-1KO mice (Drab et al., 2001) were bred at the animal facility of Dresden Technical University. The mice were genotyped before the experiment. Mice were killed by cervical dislocation. The local animal welfare officer supervised the health status, and all experiments were performed according to German legislation (permit no. 24-9168.24-1-2002-8 of the Dresden Regierungspräsidium).

Saphenous arteries
Both saphenous arteries were isolated, dissected and set up in physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, glucose 5.5 and ethylenediaminetetraacetic acid (EDTA) 0.027. After the tissues had been mounted, 1.6 mM $CaCl_2$ was added to the bath solution. Segments (2 mm long) were mounted on two wires (diameter of 40 μm) in microvascular myographs for isometric tension recordings, as described by Mulvany and Halpern (1977). One wire was attached to an isometric force transducer and the other to a displacement unit, permitting control of the internal circumference of the preparation. The organ bath contained PSS gassed with 5% CO_2 in O_2. Vessels were allowed to equilibrate in PSS at 37°C and set at a tension (T) corresponding to a pressure (P) of 70 mm Hg according to $P = T2\pi U^{-1}$ (where U is the internal circumference). This passive tension was found to be optimal for maximal force development.

Electron microscopy
For transmission electron microscopy, the mouse tissues were fixed in 2.5% glutaraldehyde (in 0.1 M sodium cacody-

late buffer, pH 7.4) overnight at 4°C. After being washed in sodium cacodylate buffer, sections were postfixed in buffered osmium tetroxide solution (1% osmium tetroxide and 0.1 M sodium cacodylate buffer, pH 7.4) for 2 h at 4°C, washed again, dehydrated in ethanol and embedded in Epon 812. Polymerization was carried out at 60°C. The specimens were viewed at 80 kV using a Zeiss EM 906 microscope (Zeiss, Oberkochen, Germany).

Measurement of vessel thickness
Vessel thickness was determined with a water immersion microscope, using a 40-times magnified scaled ocular.

Functional measurements
The viability of the preparations was examined by an initial exposure to 10 μM (−)-noradrenaline. Once contraction was stable 10 μM carbachol was added to test viability of the endothelium.

To investigate α-adrenoceptors, the vessels were exposed to the β-adrenoceptor antagonist (−)-propranolol (1 μM) and noradrenaline reuptake-inhibitor cocaine (3 μM) for at least 15 min. After this equilibrium phase the bath solution was renewed (still containing (−)-propranolol and cocaine). Concentration-effect curves for (−)-noradrenaline (10^{-11}–10^{-4} M) were determined in the absence and presence of prazosin (10^{-8}–10^{-6} M).

To investigate β-adrenoceptors, the vessels were precontracted by changing the bath solution to a PSS-60 solution, containing (mM) NaCl 63.7, KCl 60, $CaCl_2$ 1.6, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, glucose 5.5, phentolamine (10 μM) and EDTA 0.027. The vessels were incubated with β-adrenoceptor subtype-selective antagonists for at least 15 min before concentration-effect curves for (−)-isoprenaline (10^{-9}–10^{-4} M) or for the β_3-adrenoceptor-selective agonist BRL37344 were determined. The experiments were terminated with the administration of 30 μM sodium nitroprusside (SNP) to assess maximum relaxation.

Molecular biology
Saphenous arteries were isolated and frozen immediately. Total RNA was isolated using the EZNA Total RNA-Kit (Peqlab, Erlangen, Germany). Four arteries were used from two mice and pooled for mRNA isolation. The procedure was repeated three times, so that a total of six WT mice and six cav-1KO mice were used. Real-time PCR was performed using the QuantiTect SYBR Green RT-PCR kit (Qiagen, Hilden, Germany) and Rotor Gene thermal cycler (Corbett Research, Mortlake, Australia). We used the primers shown in Table 1 (Chernogubova et al., 2005). RT-PCR and real-time PCR conditions were 50°C for 30 min, 95°C for 15 min, 30 s at 94°C, 30 s at 60°C for α_{1B}, α_{1D} and β_1-adrenoceptors, respectively, and at 64°C for α_{1A}, β_2 and β_3-adrenoceptors 30 s at 72°C and 15 s at 80°C. cRNA standards were produced by T7 in vitro transcription with the Message Machine Kit (Ambion, Austin, TX, USA) from RT-PCR products produced as above, but by using a modified forward primer containing an additional T7 promoter sequence (GGCCGCGG).

Standards of known concentrations of RNA included in each dilution series (10^5–10^{12} mol μl^{-1}) allowed calculation of cRNAs. Results were analysed by Rotorgene software version 4.6 (Corbett Research, Mortlake, Australia).

Data analysis and statistics
The mechanical responses were measured as force and expressed as wall tension, ΔT, which is the increase in measured force, ΔF, divided by twice the segment length (Mulvany and Halpern, 1977). Relaxations were expressed as a percentage of the response to SNP at the end of the concentration–relaxation response curves. By using a computer programme (Graph Pad Prism, San Diego, CA, USA), the concentration–effect curves were fitted to the Hill equation and in the case of biphasic curves they were fitted with the equation for two receptor populations. Sensitivity to the agonists was expressed as –log (EC_{50}M). The results are expressed as mean $\pm$ s.e.m.. Unless stated otherwise, number of experiments are expressed as arteries from n mice. Means of multiple groups were compared by t-tests. Probability levels less than 5% were considered significant. If variances were different, Mann–Whitney test was used to test statistical significance.

Drugs
The following drugs were used: BRL37344 (GlaxoSmithKline, Harlow, Essex, UK), ICI118551 (Tocris, Bristol, UK), L748337 (Lilly, IN, USA), (−)-isoprenaline (hydrochloride), (−)-noradrenaline (bitartrate), phentolamine, CGP20712, carbachol, cocaine hydrochloride, prazosin, (−)-propranolol, ΩL-nitro-arginine and SNP were purchased from Sigma (Deisenhofen, Germany).

All drugs were dissolved in water, except (−)-noradrenaline and (−)-isoprenaline, which were dissolved in water containing 200 μM ascorbic acid and 40 μM EDTA.

Results

Carbachol-evoked relaxations
Carbachol (10 μM)-evoked relaxations of contractions induced by (−)-noradrenaline (10 μM) were not different ($P=0.59$) in WT ($51.4 \pm 3.9\%$, $n=57$ arterial segments from 18 mice) and cav-1KO ($48.7 \pm 3.1\%$, $n=85/22$) mice.

Vessel morphology
Electron microscopy revealed that vascular smooth muscle cells of WT mice are edged by caveolae (Figure 1), whereas

Table 1 Primers used for real-time PCR of adrenoceptors

| Gene	Acc. No.		Primer sequence (5'–3')	Size (bp)
Adra1a	NM_013461	Sense:	GGACAAGTCAGACTCAGAGCAAGTGACG	28
		Antisense:	TATAGCCCAGGGCATGCTTGGAAGAC	26
Adra1b	NM_007416	Sense:	TTTCATGAGGACACCCTCAGCAGTACC	27
		Antisense:	CTGCCGCTGTCATCCAGAGAGTCC	24
Adra1d	NM_013460	Sense:	GGCCAAAGGAAATCCAGGGACAC	23
		Antisense:	CAGAGCGGAACTTATGGGACAGG	23
Adrb1	NM_007419	Sense:	CGCCTGCTACAACGACCCCAAG	22
		Antisense:	AGCCAGTTGAAGAAGACGAAGAGGCG	26
Adrb2	NM_007420	Sense:	GGTTATCGTCCTGGCCATCGTGTTTG	26
		Antisense:	TGGTTCGTGAAGAAGTCACAGCAAGTCTC	29
Adrb3	NM_013462	Sense:	TCTAGTTCCCAGCGGAGTTTTCATCG	26
		Antisense:	CGCGCACCTTCATAGCCATCAAACC	25
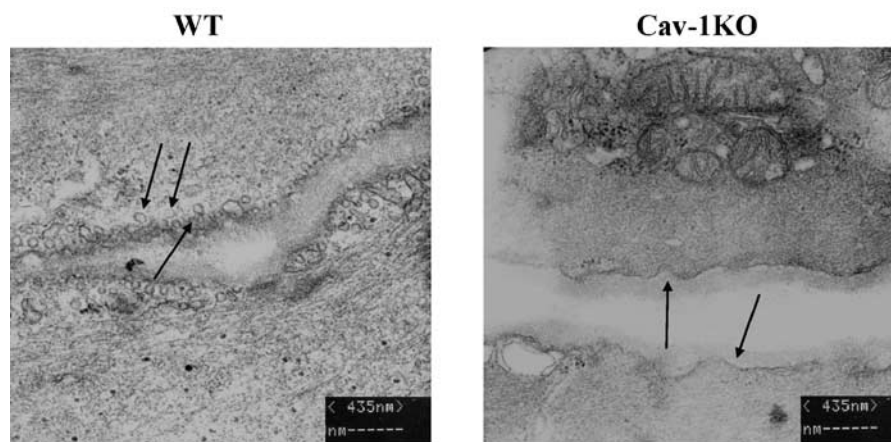
WT　　　　　　　**Cav-1KO**

Figure 1 Electron microscopy of vascular smooth muscle cells (VSMC). WT: cell membranes of VSMC of WT mice exhibit numerous invaginations (caveolae, indicated by arrows). Caveolin-1 knockout mice (Cav-1KO): cell membranes of VSMC of cav-1KO mice show shallow indentations (arrows) but no caveolae.

membranes of cav-1KO mouse cells are smooth and caveolae are absent. Thus, the interruption of only the caveolin-1 gene is sufficient to prevent the appearance of caveolae in vascular smooth muscle cells. We also observed hypertrophy of cav-1KO saphenous arteries. Arterial walls appeared 39% thicker in cav-1KO ($32 \pm 1\,\mu$m, $n = 4/2$) than in WT ($23 \pm 1\,\mu$m, $n = 4/2$) mice.

Molecular analysis
By real-time PCR, we could not find differences in expression of the mRNA levels for α_{1A}, α_{1B}, α_{1D}, β_1, β_2 and β_3

adrenoceptors between WT and cav-1KO mice (Figure 2). Interestingly, expression levels of α_{1A} mRNA were extremely small compared with α_{1B} and α_{1D}-mRNA levels. The same was found for the level of β_3-adrenoceptor mRNA, which was less than 1% of expression levels of β_1- and β_2-adrenoceptor mRNA.

(−)-Noradrenaline-evoked contractions
(−)-Noradrenaline caused concentration-dependent contractions in WT and cav-1KO arteries (Figure 3). The maximum wall tension induced by (−)-noradrenaline was significantly
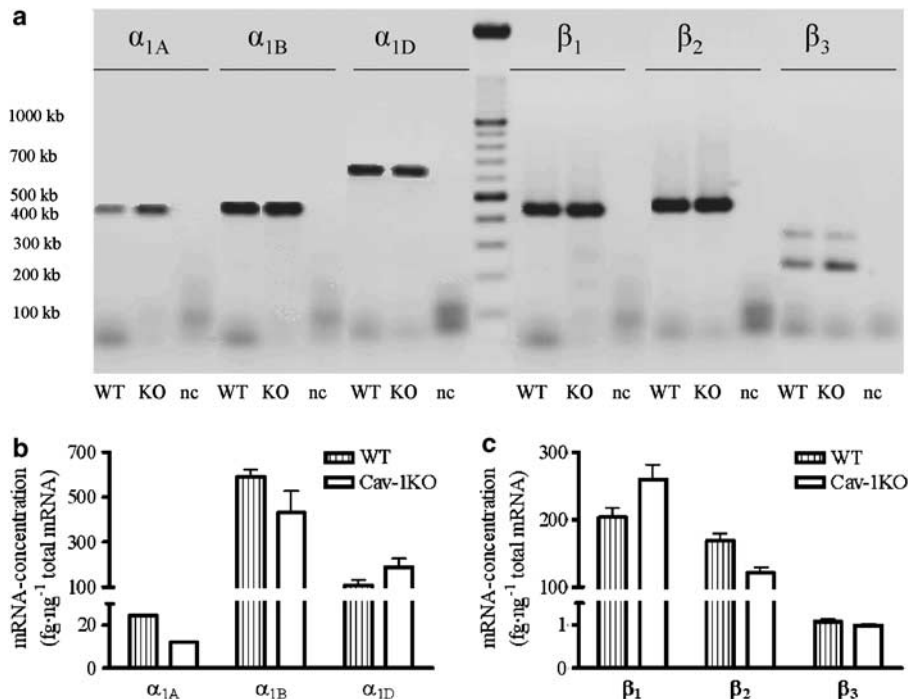
Figure 2 Expression of subunits of adrenoceptors in murine *arteria saphena* (*Aa. saphena*). Mean values from three experiments with pooled data from two mice each. (**a**) Agarose gel electrophoresis of the samples after OneStep RT real-time PCR: notice that there are two splice variants of β_3-adrenoceptor. (**b**) Amount of α-adrenoceptor-mRNA normalized to total mRNA. (**c**) Amount of β-adrenoceptor-mRNA normalized to total mRNA. Data from the two β_3-adrenoceptor splice variants were pooled.
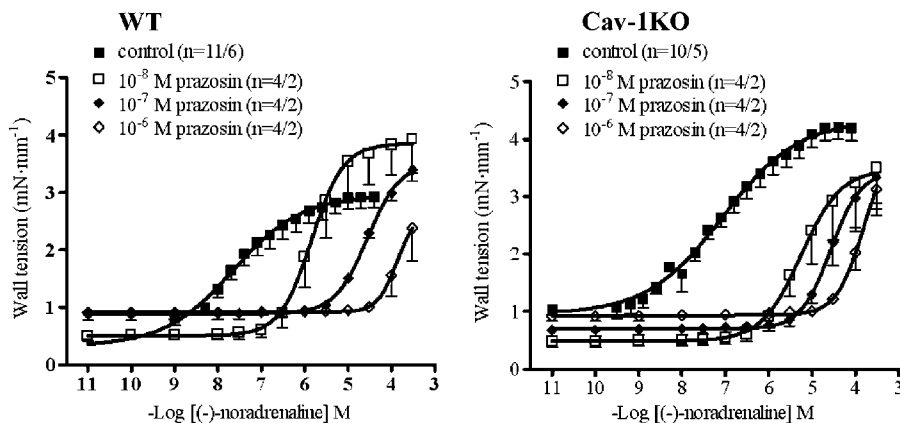
Figure 3 Concentration–effect curves for (−)-noradrenaline and antagonism by prazosin on *Aa. saphena* of WT and cav-1KO mice. Numbers refer to arterial preparations/number of mice.

higher ($P < 0.0001$; Figure 3) in cav-1KO (4.2 ± 0.2 mN mm^{-1}, $n = 10/5$) than in WT vessels (2.9 ± 0.2 mN mm^{-1}, $n = 11/6$). The concentration–effect curves for (−)-noradrenaline extended over 4 log units. Hill slopes were 0.67 ± 0.06 in WT and 0.54 ± 0.05 in cav-1KO. The potency of (−)-noradrenaline was similar for arteries of WT ($-\log EC_{50}M = 7.25 \pm 0.23$) and cav-1KO arteries ($-\log EC_{50}M = 7.10 \pm 0.12$).

Prazosin antagonized in a concentration-dependent manner the effects of (−)-noradrenaline in both WT and cav-1KO arteries (Figure 3). The curves for (−)-noradrenaline in the presence of prazosin 10^{-8} and 10^{-7} M were steeper than the curves in the absence of prazosin, with Hill slopes of 1.27 ± 0.04 and 1.25 ± 0.07, respectively. Owing to the high blocking potency of prazosin, only a partial concentration-effect curve was obtained for (−)-noradrenaline in the presence of prazosin (10^{-6} M) but the curve also tended to have a steep slope.

Effects of cocaine

Cocaine 3 μM increased the wall tension significantly stronger in arteries from cav-1KO ($53.4 \pm 2.4\%$ of the effect of (−)-noradrenaline, $n = 8/4$) than from WT ($21.7 \pm 4.1\%$, $n = 10/5$) mice (Figure 4). The effect of cocaine disappeared after washes with a PSS solution containing cocaine (Figure 4). Prazosin relaxed basal tension of arteries from cav-1KO but not from WT mice, consistent with the neuronal leak of noradrenaline; prazosin also prevented cocaine-induced contractions (results not shown, $n = 4/2$).

SNP-evoked relaxations

Maximum relaxation was determined with 30 μM SNP. Wall tension at 60 mM KCl was significantly greater in WT (2.99 ± 0.16 mN mm^{-1}, $n = 43/27$) than in cav-1KO (2.18 ± 0.12 mN mm^{-1}, $n = 52/30$) vessels. There was no difference in percentage relaxation caused by SNP between both groups ($58 \pm 2\%$ for WT and $57 \pm 2\%$ for cav-1KO, Figure 5).

(−)-Isoprenaline-evoked relaxations

Vascular relaxation, mediated through β-adrenoceptors, was investigated with (−)-isoprenaline on arteries precontracted with 60 mM KCl. (−)-Isoprenaline relaxed arteries of cav-1KO and WT mice with similar maximum effects (E_{max}) but lower potency on cav-1KO than on WT ($P < 0.03$) (Table 2: mean
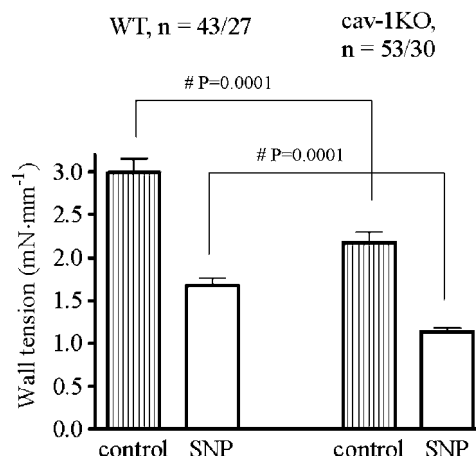

Figure 5 Relaxant effects of SNP (30 μM) on *Aa. saphenae* precontracted with 60 mM KCl. Numbers refer to arterial preparations/number of mice.
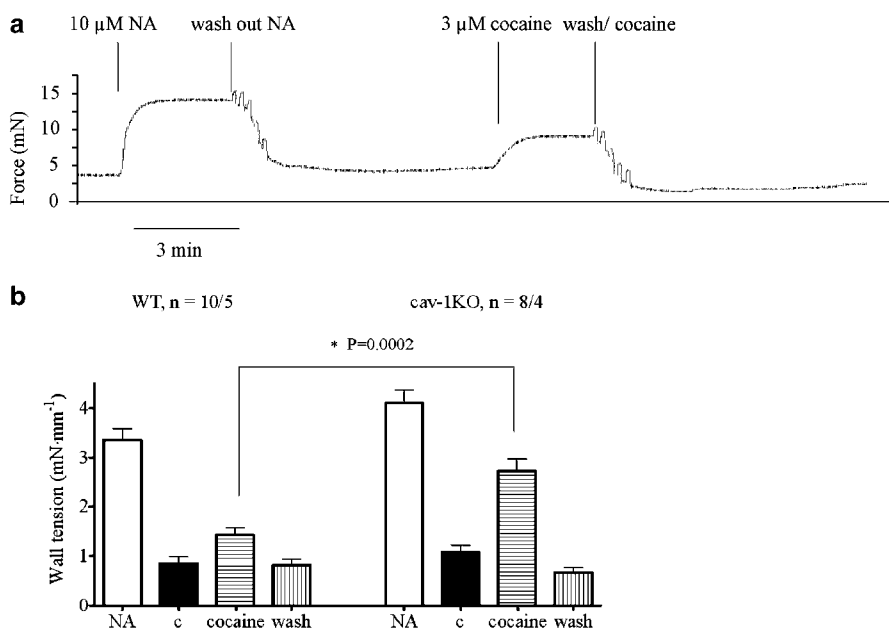
Figure 4 Effects of cocaine on *Aa. Saphenae* of WT and cav-1KO mice. (**a**) Original tracing of an experiment on a cav-1KO artery. (**b**) Addition of 3 μM cocaine leads to contraction of the vessels. This effect is significantly stronger in cav-1KO compared with WT mice ($P = 0.0002$). In both groups of arteries, the effect was washed out with PSS containing 3 μM cocaine. c = control; NA = (−)-noradrenaline (10 μM). Numbers refer to arterial preparations/number of mice.

Table 2 Effect of β-AR antagonists on relaxation curves of (−)isoprenaline

		WT					*KO*			
	n	$-\log EC_{50}M$	P	E_{max}	P	n	$-\log EC_{50}M$	P	E_{max}	P
(−)-Isoprenaline										
Control	13/7	7.31 ± 0.17		39.9 ± 6.3		12/7	6.84 ± 0.1		36.7 ± 3.4	
CGP20712A (300 nM)	8/4	6.93 ± 0.29	NS	19.6 ± 3.7	<0.02	6/3	7.21 ± 0.06	<0.03	51.3 ± 2.9	<0.02
ICI118551 (50 nM)	5/3	6.74 ± 0.1	<0.04	28.7 ± 4.2	NS	6/3	6.21 ± 0.09	<0.002	29.7 ± 4.5	NS
L748337 (100 nM)	4/2	6.62 ± 0.34	NS	33.3 ± 6.3	NS	10/5	6.85 ± 0.07	NS	42.4 ± 4.7	NS
CGP20712A + ICI118551	8/4	5.24 ± 0.12	<0.0001	12.8 ± 2.1	<0.002	7/4	7.99 ± 0.20^{H}	<0.0001	8.2 ± 2.0	<0.0001
						7/4	5.32 ± 0.10^{L}	<0.0001	36.0 ± 3.4	<0.0001
CGP20712A + ICI118551 + L748337	3/2	5.10 ± 0.46	<0.0004	25.4 ± 8.8	NS	5/4	5.22 ± 0.25	<0.0001	17.0 ± 7.9	<0.02
BRL37344										
Control	3/2	5.18 ± 0.41		8.6 ± 4.5		7/4	8.73 ± 0.35^{H}		6.25 ± 2.5	
						7/4	6.10 ± 0.18^{L}		19.4 ± 3.5	
L748337 (100 nM)						7/4	6.51 ± 0.45	NS	18.9 ± 4.3	NS
Control (L-NAME experiments)						6/3	8.75 ± 0.09^{H}		26.9 ± 2.5	
						6/3	6.22 ± 0.38^{L}		64.5 ± 2.8	
L-NAME (100 μM)						6/3	8.29 ± 0.27^{H}	NS	15.8 ± 1.7	<0.04
						6/3	5.74 ± 0.33^{L}	NS	39.2 ± 4.0	<0.03

Abbreviation: NS, not significant.
P-values are with respect to the corresponding control in the WT and cav-1KO.
E_{max}: Maximum relaxation in the presence of the agonist compared with relaxation in the presence of 30 μM SNP.
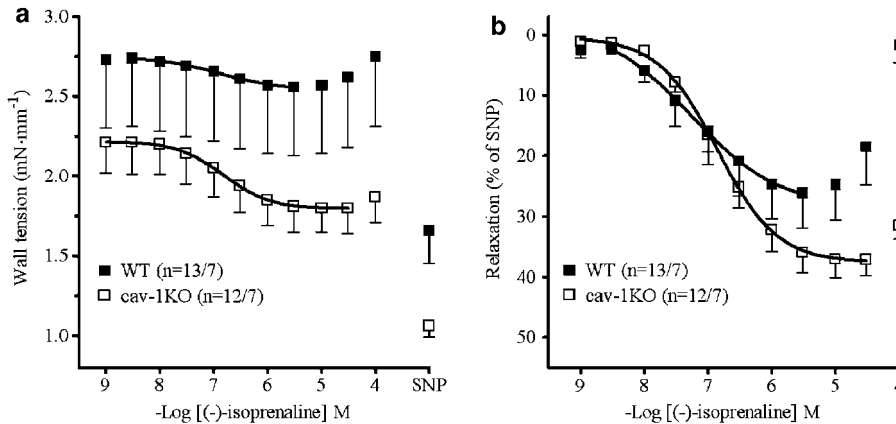[H]: high-affinity component.
[L]: low-affinity component.

Figure 6 Concentration–effect curves of (−)-isoprenaline in *Aa. saphenae* of WT and cav-1KO mice. (**a**) Curves showing reduction in wall tension. (**b**) Curves normalized to SNP-evoked relaxation. Numbers refer to arterial preparations/number of mice.

E_{max} values from curves fitted to single experiments; Figure 6: average curve).

The β_1-adrenoceptor-selective antagonist CGP20712A (300 nM) did not significantly affect the potency of (−)-isoprenaline but reduced the E_{max} in WT arteries (Figure 7). In vessels of cav-1KO mice, CGP20712 paradoxically appeared to slightly enhance E_{max} and to shift the concentration–relaxation curve of (−)-isoprenaline to the left (Table 2: mean E_{max} values from curves fitted to single experiments; Figure 7: average curve). The different effects of CGP20712 on the responses to (−)-isoprenaline appeared unrelated to the age of the mice, which was 80 weeks for WT and 74 weeks for cav-1KO.

The β_2-adrenoceptor-selective antagonist ICI118551 (50 nM) shifted concentration–relaxation curves of (−)-iso-

prenaline similarly to the right in WT and in cav-1KO arteries (Figure 7). The β_3-adrenoceptor-selective antagonist L748337 (100 nM) did not significantly affect the con-centration–relaxation curves of (−)-isoprenaline in WT and cav-1KO arteries (Figure 7).

The combination of CGP20712A (300 nM) and ICI118551 (50 nM) caused a greater shift of the concentration–relaxation curve to (−)-isoprenaline of WT arteries than each antagonist, administered separately (Figure 8). The corresponding curve of cav-1KO arteries was shifted less to the right than with the WT arteries and the curve was biphasic. The different effects of the combination of CGP20712 and ICI118551 on the responses to (−)-isoprenaline appeared to be unrelated to the age of the mice, which was 80 weeks in WT and 75 weeks in cav-1KO. The high-affinity (H)
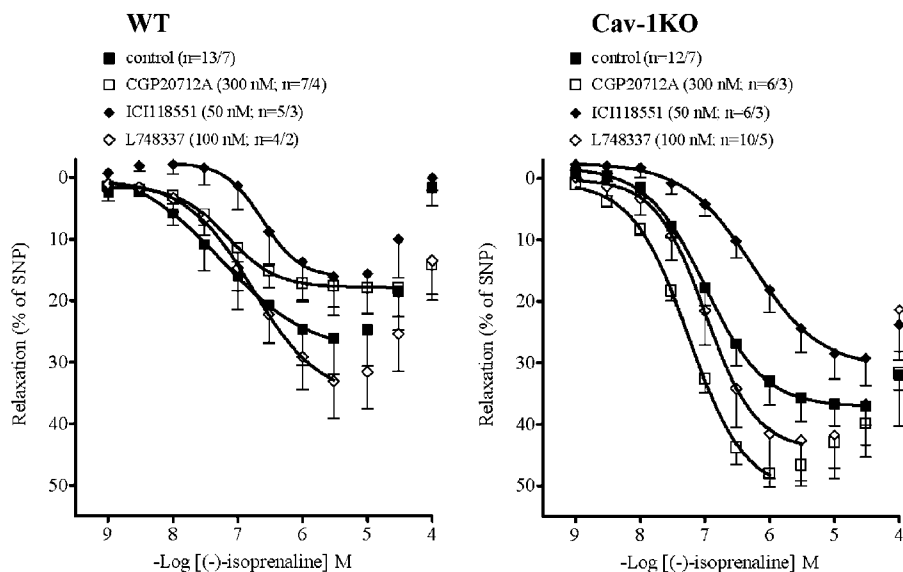
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Figure 7 Comparison of the effects of β-blockers on (−)-isoprenaline-evoked relaxation in WT and cav-1KO arteries. For –log EC_{50}M and E_{max} data, see Table 2.
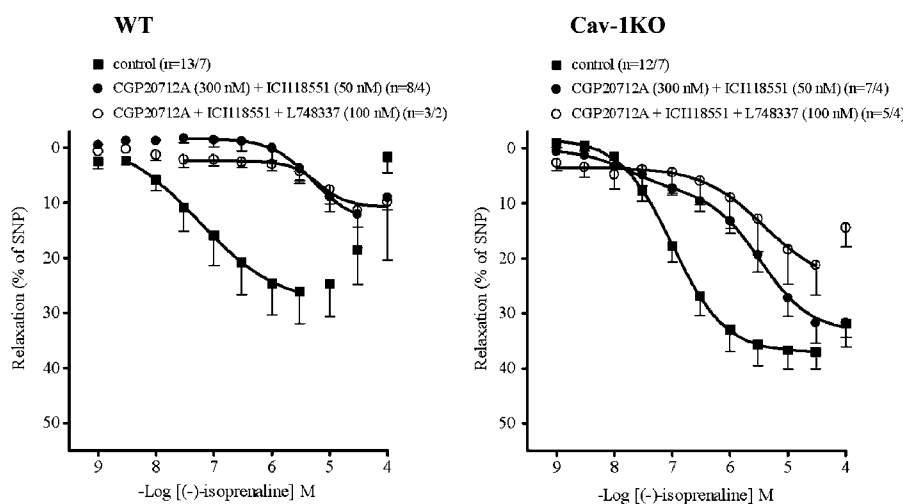

Figure 8 Comparison of the effects of β-blocker combinations on (−)-isoprenaline-evoked relaxation in WT and cav-1KO arteries. For –log EC_{50}M and E_{max} data, see Table 2.

component of the relaxant effects of (−)-isoprenaline, resistant to blockade by the combination of CGP20712 and ICI118551, amounted to 23% (Table 2). The H component was suspected to be mediated through β_3-adrenoceptors. As expected, the β_3-selective antagonist L748337 (100 nM), in the presence of both CGP20712 and ICI118551, shifted the concentration-relaxation curve for (−)-isoprenaline further to the right, and the curve became monophasic (Table 2 shows mean E_{max} values from curves fitted to single experiments; Figure 8 shows average curve).

BRL37344-evoked relaxation
To investigate the involvement of β_3-adrenoceptors further in the (−)-isoprenaline-evoked relaxation in cav-1KO arteries, the β_3-selective agonist BRL37344 was used. In arteries from

WT mice, BRL37344 only caused relaxation at concentrations greater than 1 μM, suggesting mediation through β_1 and/or β_2-adrenoceptors (Figure 9a). In arteries from cav-1KO mice, the concentration–relaxant curve for BRL37344 was biphasic with a high-affinity (H, nM) component and low-affinity component (L, μM) (Figure 9b, Table 2). L748337 (100 nM) blocked the H component, consistent with mediation through β_3-adrenoceptors, but did not affect the L component (Table 2 shows mean E_{max} values from curves fitted to single experiments; Figure 9 shows average curve).

To determine whether the BRL37344-evoked relaxation was through β_3-adrenoceptors located in endothelial cells, or smooth muscle cells we used the NO synthase inhibitor ΩL-nitroarginine (L-NAME). The concentration–effect curve for BRL37344 was not shifted by L-NAME (Figure 9b). The curves for BRL37344 were biphasic both in the absence and
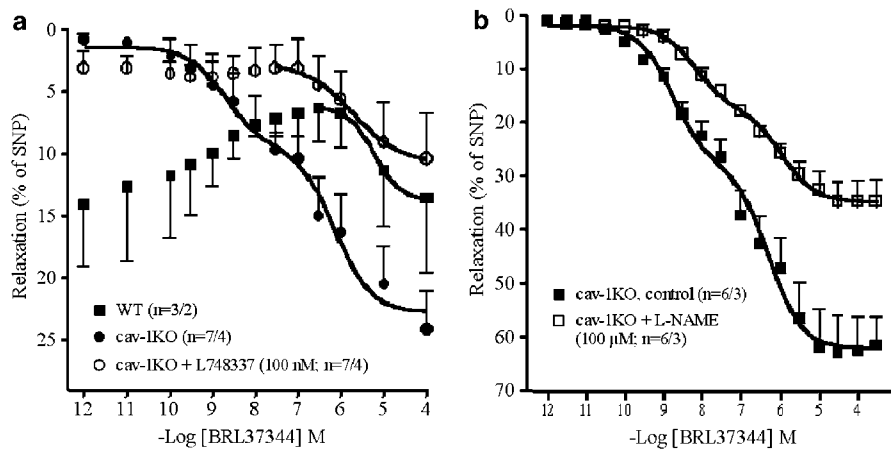
Figure 9 Concentration-effects curves for the β_3-adrenoceptor-selective agonist BRL37344. (**a**) Antagonism of BRL37344-evoked relaxations by L748337 in cav-1KO. (**b**) Partial reduction of the relaxant effects of BRL37344 by L-NAME. For $-\log EC_{50}M$ and E_{max} data, see Table 2.

presence of L-NAME. The $-\log EC_{50}M$ values for the H and L component of the BRL37344 response were not significantly changed by L-NAME (Table 2) but E_{max} was reduced significantly in the presence of L-NAME (Table 2). For unknown reasons, the experiments with BRL37344 in the absence of L-NAME (Figure 9b) showed stronger relaxation responses than the experiments depicted in Figure 9a, but their $-\log EC_{50}M$ for the corresponding H and L components were not significantly different.

Discussion

The murine saphenous artery contracts in response to (−)-noradrenaline through activation of α_1-adrenoceptors and relaxes in response to (−)-isoprenaline, mainly through activation of β_2-adrenoceptors and to a moderate extent through β_1-adrenoceptors. The absence of caveolae in arteries of cav-1KO mice does not reduce either α_1-adrenoceptor-mediated contractions or β_2-adrenoceptor-mediated relaxations, but abolishes β_1-adrenoceptor-mediated relaxations and causes the appearance of β_3-adrenoceptor-mediated relaxations. Thus, unlike β_2-adrenoceptor-mediated relaxation, β_1-adrenoceptor-mediated arterial relaxation requires intact caveolae.

Similar carbachol-induced relaxations in cav-1KO and WT
The interaction of the caveolin-1 scaffolding domain (CSD) and endothelial nitric oxide synthase (eNOS) inhibits eNOS activity (Ju *et al.*, 1997). Injection of a peptide containing the CSD inhibits both acetylcholine-evoked vasodilatation of murine aortae and NO production (Bucci *et al.*, 2000). As expected, NO production and acetylcholine-evoked relaxation of aortae are enhanced in cav-1KO mice (Drab *et al.*, 2001; Razani *et al.*, 2001). However, the carbachol-induced relaxation was not different in saphenous arteries of WT and cav-1KO mice, which does not accord with an enhanced release of NO. The reasons for this discrepancy between murine aorta and saphenous artery are not known.

(−)-Noradrenaline-evoked contractions are increased in cav-1KO
The walls of cav-1KO saphenous arteries showed smooth muscle hypertrophy compared with WT arteries. Wall hypertrophy could explain the greater maximal contractions produced by (−)-noradrenaline in cav-1KO than in WT arteries. However, for unknown reasons, 60 mM KCl contractures were decreased in cav-1KO, implying that (−)-noradrenaline and KCl increase vascular tension through different mechanisms. Prazosin caused concentration-dependent and similar antagonism of the effects of (−)-noradrenaline in cav-1KO and WT arteries. The steepening of the concentration–effect curves by prazosin suggests involvement of more than one α-adrenoceptor subtype and precluded a quantitative assessment of the affinity for the α_1-adrenoceptors.

Our finding of enhanced noradrenaline-evoked contractions, mainly mediated through α_1-adrenoceptors, of the saphenous artery in cav-1KO is at variance with previous work. Cav-1KO aortae have been found to exhibit blunted responses to phenylephrine (Drab *et al.*, 2001; Razani *et al.*, 2001). These authors attributed the blunted phenylephrine-induced contractions to an increase in constitutive activity of eNOS (and hence NO release), owing to the lack of constrain of this enzyme by caveolin-1 in cav-1KO. However, our results showing unaltered carbachol-induced relaxations in cav-1KO compared with WT suggest that this interpretation does not apply to the murine saphenous artery.

Cocaine-evoked contractions are increased in cav-1KO
The increased cocaine-evoked contractions in cav-1KO, compared with WT arteries, appear to be at least partially the result of vascular hypertrophy. Interestingly, the effect of cocaine could be washed out with PSS containing cocaine, making it unlikely that it has a direct interaction with the muscle cells. Noradrenaline may leak out from adventitial nerve terminals but partially be recaptured again by the nerves. The cocaine-evoked contractions could be the result of the prevention of neuronal reuptake, thereby making more (−)-noradrenaline available to the

α_1-adrenoceptors (De la Lande and Waterson, 1967). For unknown reasons, the postulated (−)-noradrenaline leakage could also be more pronounced in arteries from cav-1KO than from WT mice.

Abolished β_1-adrenoceptor-mediated relaxation in cav-1KO

The relaxant potency of (−)-isoprenaline was slightly lower in cav-1KO than in WT, presumably owing to the loss of a β_1-adrenoceptor-mediated component. The β_1-adrenoceptor-selective antagonists CGP20712 reduced the E_{max} of (−)-isoprenaline-evoked relaxation in arteries from WT mice, but not from cav-1KO mice. For unknown reasons CGP20712 actually tended to enhance the relaxant responses to (−)-isoprenaline in cav-1KO. Nevertheless, the result suggests the existence of a β_1-adrenoceptor component of (−)-isoprenaline-induced relaxation mediated through β_1-adrenoceptors in WT but not in cav-1KO. Thus, caveolae appear to be essential for the manifestation of the β_1-adrenoceptor component. This is a somewhat unexpected finding because, unlike cardiac β_1-adrenoceptors, β_1-adrenoceptors of rat aorta smooth muscle do not appear to be associated with caveolar membrane fractions (Ostrom et al., 2002). It seems that caveolae and caveolin-1 facilitate either the expression or the coupling of β_1-adrenoceptors to the biochemical effectors leading to vascular relaxation. As β_1-adrenoceptor mRNA levels were not different in arteries of WT and cav-1KO mice, it seems more likely that the absence of caveolae may prevent β_1-adrenoceptor coupling through, as yet, unknown mechanisms.

G protein-coupled receptor kinases (GRKs) phosphorylate and desensitize G-protein coupled receptors, and it has been shown that GRK2-catalysed phosphorylation is inhibited by caveolin-1 (Carman et al., 1999). Conceivably, the lack of the caveolin-1 constraint in cav-1KO mice liberates GRK2 to phosphorylate β_1-adrenoceptors of the murine saphenous artery more quickly, thereby partially preventing the (−)-isoprenaline-evoked relaxation, but this plausible mechanism requires further research.

Preserved function of vascular β_2-adrenoceptors in cav-1KO mice

(−)-Isoprenaline relaxes the murine saphenous artery mainly through β_2-adrenoceptors in both WT and cav-1KO mice. This finding is consistent with the lack of caveolar localization of β_2-adrenoceptors in the smooth muscle of rat aorta (Ostrom et al., 2002). These results are also consistent with our data demonstrating similar β_2-adrenoceptor mRNA levels in saphenous arteries from WT and cav-1KO mice.

β_3-Adrenoceptors substitute for β_1-adrenoceptors to mediate relaxation in cav-1KO mice

Both (−)-isoprenaline and the β_3-adrenoceptor-selective agonist BRL37344 partially relaxed the saphenous artery in cav-1KO but not in WT mice. Their effects were antagonized by the β_3-adrenoceptor-selective antagonist L748337, consistent with mediation through β_3-adrenoceptors. Although real-time PCR did not reveal quantitative differ-

ences in mRNA expression of β_3-adrenoceptors, the receptors only mediated relaxation in arteries from cav-1KO but not from WT mice. The two murine splice variants β_{3a} and β_{3b}, reported by Evans et al. (1993), were found apparently unaltered (Figure 2a). The unaltered mRNA levels suggest that the relaxant β_3-adrenoceptor function is suppressed by caveolae through an unknown mechanism in WT arteries, either through lack of expression of sufficient β_3-adrenoceptors or through uncoupling of these receptors or both.

The β_3-adrenoceptors seem to replace the disappeared relaxant function of β_1-adrenoceptors in saphenous arteries of cav-1KO mice. Both endothelial and smooth muscle β_3-adrenoceptors can contribute to vascular relaxation. Endothelium-independent relaxations have been found in human placental arteries (Rouget et al., 2006) and corpus cavernosum (Cirino et al., 2003), canine pulmonary artery (Tamaoki et al., 1998), rat abdominal aortae (Matsushita et al., 2003) and carotid arteries (Oriowo, 1994). Endothelium-dependent relaxations appear to occur through β_3-adrenoceptors in human mammary artery (Rozec et al., 2005), rat thoracic aorta (Trochu et al., 1999) and microvascular bed of human coronary artery (Dessy et al., 2005). In the latter system, (−)-isoprenaline relaxes the vessels through both the release of NO and a hyperpolarizing factor (Dessy et al., 2005).

Our results, showing that ΩL-nitroarginine reduces but does not abolish the H component of BRL37344-evoked relaxation, suggest that the β_3-adrenoceptors that appeared in cav-1KO may be located on both endothelial cells and smooth muscle cells.

Unlike β_1- and β_2-adrenoceptors, the β_3-adrenoceptor has fewer phosphorylation sites and is fairly resistant to desensitization (Liggett et al., 1993; Jockers et al., 1996). Therefore, prolonged agonist exposure, acting through β_3-adrenoceptors of human myometrium, does not result in desensitization (Rouget et al., 2004). For the same reason, the β_3-adrenoceptor-mediated relaxation of the cav-1KO saphenous artery remains apparent, unlike the β_1-adrenoceptor-mediated relaxation, because this receptor could be phosphorylated by the unconstrained GRK2 activity, as discussed above.

Conclusions

We conclude that the function α_1-adrenoceptors and β_2-adrenoceptors is preserved in saphenous arteries from cav-1KO mice devoid of caveolae. However, the β_1-adrenoceptor-mediated vascular relaxation, observed in WT mice, is abolished in cav-1KO mice, pointing to an essential role of caveolae. The disappearance of β_1-adrenoceptor function is compensated by the appearance of functional β_3-adrenoceptors, probably located on both endothelial cells and vascular smooth muscle cells in cav-1KO mice.

Conflict of interest

The authors state no conflict of interest.

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